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# Accepted Manuscript

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# TRPA1 acts in a protective manner in imiquimod-induced psoriasiform dermatitis in mice

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Running title: TRPA1 enhances the imiquimod-induced psoriasiform skin inflammation

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**Abstract**

The present study revealed the modulatory role of Transient Receptor Potential Ankyrin 1 (TRPA1) and Vanilloid 1 (TRPV1) cation channels in Aldara-induced (5% imiquimod, IMQ) murine psoriasis model using selective antagonists and genetically altered animals. We have also developed a refined localized model to enable internal controls and reduce systemic effects. Skin pathology was quantified by measuring skin thickness, scaling, blood flow and analyzing dermal cellular infiltrate, while nocifensive behaviours were also observed. Cytokine gene expression profiles were measured *ex vivo*. Psoriasiform dermatitis was significantly enhanced in TRPA1 KO mice and with TRPA1 antagonist (A967079) treatment. By comparison, symptoms were decreased when TRPV1 function was inhibited. IMQ induced  $\text{Ca}^{2+}$  influx in TRPA1-, but not in TRPV1-expressing cell lines. Immunohistochemical studies revealed that CD4+ T helper cells express TRPA1 but not TRPV1 ion channels in mice skin. Compared to the TRPV1 KO animals, additional elimination of the TRPA1 channels in the TRPV1/TRPA1 double KO mice did not modify the outcome of the IMQ-induced reaction, further supporting the dominant role of TRPV1 in the process. Our results suggest that the protective effects in psoriasiform dermatitis can be mediated by the activation of neuronal and non-neuronal TRPA1 receptors.

## **Introduction**

Psoriasis is a chronic, recurrent immune-mediated inflammatory skin disease, affecting 2-3% of the population. The most common type of the disease is *psoriasis vulgaris* that occurs in about 90% of the patients. Clinically, the disease is characterized by sharply demarcated, scaly, erythematous skin lesions, and pruritus (Nestle et al., 2009). It is widely accepted that T lymphocytes play a key role in psoriasis, as they chronically colonize the skin and promote the proliferation of keratinocytes. T cell activation is initiated and driven by dendritic cells that, on onset or exacerbation of psoriasis, produce various pro-inflammatory cytokines, including TNF- $\alpha$  and IL-23. The critical roles for immune cells and cytokines in psoriasis pathogenesis is supported by the observation that treatments targeting the immune system, such as antibodies against TNF- $\alpha$ , IL-12/23 or IL-17, are highly effective in improving the disease (Nestle et al., 2009).

Imiquimod (IMQ)-induced psoriasiform skin inflammation in mice is the most frequently used animal model to study the pathomechanism of psoriasis (van der Fits et al., 2009; Swindell et al., 2017). The IMQ model recapitulates many of the phenotypic changes of psoriasis and mimics its IL-17/IL-23-driven pathology. IMQ acts primarily via the ligation of TLR7 (in mice and human) or TLR8 (only in human) (Hemmi et al., 2002; Jurk et al., 2002), although a TLR7-independent mechanism has also been proposed (Kanneganti et al., 2006; Schön et al., 2006). Despite some controversy, the roles for cytokines in this model, especially TNF- $\alpha$  and the IL-17/IL-23 axes, have been shown (Van Belle et al., 2011; Vinter et al., 2016).

Recently, Riol-Blanco and co-workers provided evidence that the desensitization of NaV1.8+/TRPV1+ nociceptive sensory neurons by resiniferatoxin (RTX) pre-treatment significantly decreased the inflammatory reaction in IMQ-induced psoriasiform dermatitis in

mice (Riol-Blanco et al., 2014) by blocking of IL-23 production in dermal dendritic cells (DDC). Since TRPA1 and TRPV1 non-selective cation channels are expressed on the sensory nerve endings, RTX pre-treatment causes selective elimination of the functions of these neuronal receptors. Nevertheless chemical denervation by RTX does not influence mRNA and protein expression of non-neuronal TRPV1 and TRPA1 (Kun et al., 2012). In addition, these TRP channels have previously been shown to play important roles in pruritus (Fernandes et al., 2013; S. R. R. Wilson et al., 2013) and contact dermatitis (Liu et al., 2013). Hence, this study aimed to investigate the role for TRPA1 and TRPV1 in the IMQ model of psoriasis.

## **Results**

### *Loss of TRPA1 function enhances IMQ-induced psoriasiform skin inflammation*

Clinical signs of psoriasis such as skin thickening and scaling were consistently observed in IMQ- but not vaseline-treated skin using the 2 different disease induction techniques in both male and female mice (Fig. 1 a,b, suppl Fig. S1). Dorsal skin erythema, one of the main clinical signs of psoriasis, was assessed using laser Doppler blood flowmetry technique. Measurement of dorsal skin blood flow quantitatively in this model to our knowledge is previously unreported. A significant increase in dorsal skin blood flow was observed using both IMQ treatment techniques, reaching maximal responses after the last treatment (Fig. 1 g,h), highlighting a useful application for the laser Doppler technique to assess the dorsal skin erythema in a quantitative manner. Both ‘the whole back model’ and the ‘localized model’, where IMQ application in Finn chamber technique was used, resulted in similar trends of skin thickening and scaling (Fig. 1 d,e,j,k). Thus the localized model is a to our knowledge previously unreported way of inducing skin inflammation with less systemic side effects, as indicated by the development of reduced splenomegaly (Suppl. Fig. S2).

Interestingly, TRPA1 KO mice showed exacerbated skin pathology in both disease induction models, with increased skin thickness and enhanced erythema from day 2 onwards (Fig. 1). This effect was independent of gender differences, systemic inflammatory effects, highlighting a specific, localized protective role for TRPA1 in this model (Suppl. Fig. S1, S2). To eliminate the possibility of compensatory mechanisms in the KO animals, experiments involving pre-treatment with the selective TRPA1 antagonist A967079 were also carried out. Twice daily application of A967079 over 4 days, substantially enhanced IMQ-mediated skin thickening and scaling in WT mice similar to the observed profile in TRPA1 KO mice (Fig. 1 c,f,i). In contrast to the observation in TRPA1 KO mice, A967079 treatment did not markedly influence blood perfusion response in the IMQ-treated TRPA1 WT groups (Fig. 1 i).



*Histological analysis confirms the protective role for TRPA1 in IMQ-mediated psoriasiform dermatitis*

The typical histological hallmarks of human psoriasis (keratinocyte hyperproliferation, hyperkeratosis, parakeratosis, Munro's microabscesses) were observed in IMQ-treated TRPA1 WT and KO mice. Infiltration of inflammatory cells (e.g. lymphocytes, granulocytes, macrophages) could be observed in the epidermal and dermal layers of the treated skin (Fig. 2 panel A and Suppl. Fig. S3). Accumulation of neutrophils in the epidermis resulted in Munro's microabscesses following IMQ treatment (Fig. 2 panel a). Histopathological scoring assessing characteristic parameters in psoriasis (Munro's microabscesses, epidermal thickness, and cell layers) were significantly enhanced in IMQ-treated skin samples of TRPA1 KO or A967079-treated mice compared to WT samples. (Fig. 2 b,c).

*TRPA1 KO and TRPA1 antagonist-treated mice exhibit significantly increased nocifensive behaviour*

The effect of IMQ on nocifensive and itch behaviour was determined using the 'whole back model'. The trends for hind paw scratching were inconsistent and highly variable in both TRPA1 WT and KO mice (Fig. 3 b). However, IMQ resulted in significantly increased biting/licking and flinching responses (Fig. 3 c,d). Consistent with worsening skin pathology, deletion, or long term inhibition of TRPA1 function showed trends of increased biting/licking and flinching, reaching significance on day 4 (Fig. 3). A similar profile was observed in female mice (Suppl. Fig. S4).

*Temporal inflammatory cytokine mRNA expression in TRPA1 WT and KO mice*

IMQ induced a marked increase in the mRNA expression of IL-1 $\beta$ , TNF- $\alpha$ , IL-23, IL-17, and IL-22 in WT mice, in agreement with previous publications (van der Fits et al., 2009; Flutter and Nestle, 2013; Riol-Blanco et al., 2014) (Fig. 4). TNF- $\alpha$  and IL-23 mRNA levels peaked at 6 hours (Fig. 4 b,c), while for IL-1 $\beta$ , IL-17, and IL-22 the highest mRNA expression was observed at 48 hours (Fig. 4 a,d,e). After 96 hours, all of the cytokines mRNA levels started to return to baseline (data not shown). A significantly increased expression of IL-1 $\beta$ , TNF- $\alpha$  and IL-22 mRNA was detected in the TRPA1 KO compared to WT mice, reaching maximal differences at 48 hrs. The trends of cytokines expression reflect the worsening skin pathology in TRPA1 KO mice, which is initiated as early as 6 hours following the first IMQ application.

#### *Influence of TRPV1 on the protective role of TRPA1 in IMQ response*

To determine potential interactions between TRPV1 and TRPA1 receptors in this model, we induced skin inflammation in TRPV1/TRPA1 double KO animals (Fig. 5 e,f). Double TRPA1/V1 KO led to an inhibition of skin fold thickness and reduced dorsal skin perfusion (Fig. 5 e,f). A similar tendency was also observed when TRPA1 KO mice were treated with a TRPV1 antagonist (SB366791). The enhanced skin pathology and dorsal skin erythema phenotype was inhibited with SB366791 treatment (Fig. 5 c,d). TRPV1 KO mice also showed improved skin pathology and showed trends of reduced erythema in comparison to WT mice (Fig. 5 a,b). Hence, these results suggest an opposing interaction between TRPA1 and TRPV1 in the IMQ model.

#### *Effect of IMQ in TRPA1 or TRPV1 expressing CHO, HaCaT and HEK293T cell lines*

To investigate if IMQ could directly activate TRPA1 or TRPV1 receptors or keratinocytes, we used radioactive  $^{45}\text{Ca}^{2+}$  uptake studies with TRPA1 or TRPV1 expressing CHO cell lines and HaCaT cells, a human cell line derived from keratinocytes. IMQ induced  $\text{Ca}^{2+}$  influx dose

dependently (with an EC<sub>50</sub> of 4.03  $\mu$ M) in TRPA1 expressing but not in TRPV1 expressing CHO cells. Only a low level of Ca<sup>2+</sup> influx was detected in HaCaT cells even at 100  $\mu$ M IMQ (Fig. 5 g). To ensure that the observed effect was TRPA1-dependent, in subsequent experiments varying doses of TRPA1 antagonist (A967079) were coadministered with the IMQ treatment. A967079 dose-dependently inhibited Ca<sup>2+</sup> influx in TRPA1 expressing cells in response to IMQ, highlighting that IMQ specifically activates TRPA1 (Fig. 5 h). IMQ induced a mainly outwardly rectifying transmembrane current on HEK293T cells overexpressing recombinant human TRPA1, which was abolished in the presence of HC030031, a potent and selective inhibitor of TRPA1 measured with patch clamp technique (Suppl. Fig. S5).

#### *Expression of TRPA1, but not TRPV1 receptors on CD4<sup>+</sup> T- cells*

Using confocal microscopy of IMQ-treated skin, we observed TRPA1+ staining, overlapping with CD4<sup>+</sup> expression on dermal CD4<sup>+</sup> T cells. TRPA1 was mainly expressed on the plasma membrane of CD4<sup>+</sup> T cells (Fig. 6 left panel). Intriguingly, there were no TRPV1 and CD4 co-localization in mouse dorsal skin sections (Fig. 6 right panel).

## **Discussion**

IMQ-induced skin inflammation is widely accepted as a murine model for psoriasis (Flutter and Nestle, 2013). Indeed, we observed similar degrees of psoriasiform dermatitis, *in vivo* and histopathologically, using 2 different techniques of IMQ application. We have characterized a ‘localized model’ involving Finn chamber application technique, in which both IMQ and vaseline treatment were applied on the dorsal skin of the same mouse; thus allowing lesional and non-lesional samples to be obtained from the same mouse. In this model, involving lower total IMQ levels, mice showed reduced splenomegaly, indicating less systemic inflammatory

component. The overall effect of IMQ activation results in the production of various pro-inflammatory cytokines, such as TNF- $\alpha$  (Perera et al., 2012; Quivy and Van Lint, 2004) and IL-1 $\beta$  (Kanneganti et al., 2006), while the activation of T-cells leads to the production of IL-17/IL-22/IL-23 (Van Belle et al., 2011; van der Fits et al., 2009). Our current observations confirmed the upregulation of these cytokines within 48 hours in our 'localized model', similar to previous publications (van der Fits et al., 2009). Hence, this is a suitable model for studying IMQ-induced psoriasis.

Recently, Riollot-Blanco et al used resiniferatoxin (RTX) pretreatment as an established technique to selectively deplete peripheral sensory nerve endings. Since TRPA1 and TRPV1 are co-expressed on these neurons (Kobayashi et al., 2005), this technique eliminates both receptors from neuronal elements, but has no effect on non-neuronal receptor structures (Kun et al., 2012). This leads to the following assumptions: 1. Activation of TRP or TLR-7 receptors by IMQ on non-neuronal cells is not capable to trigger psoriasiform dermatitis 2. The peripheral nociceptors/sensory nerve endings are inevitable for the development of the IMQ-induced inflammation via the activation of dermal DCs, which then release IL-23.

Here, we investigated the role of TRP channels in this process by using various TRP knockout mice. Interestingly, TRPV1 KO mice showed similar improvement as the RTX treated animals, suggesting that the presence of TRPV1 receptors is essential on the peripheral nociceptors for the IMQ-induced psoriasis. However, we have shown that this could not be a direct effect of IMQ via the TRPV1 receptor as IMQ does not activate TRPV1 expressing CHO cells. Hence, we propose that TRPV1 receptor is activated by an as yet unknown mediator released by other cells (e.g. keratinocytes) in response to IMQ. In contrast, additional elimination of the TRPA1 channels in the TRPV1/TRPA1 double KO mice did not modify the outcome of the IMQ-induced pathology, further supporting the dominant role of TRPV1 in the process. Surprisingly, when TRPV1 is intact, genetic deletion or

pharmacological blockade of TRPA1 led to a significantly enhanced IMQ-induced pathology, suggesting that under normal circumstances TRPA1 down-regulates the inflammatory process. Here we have provided evidence that the expression of Iba-1 mRNA, a marker of the activation of macrophages in the DRG, is significantly elevated after IMQ treatment in TRPA1 KO mice compared to wild type animals (see Suppl. Fig S6). It is possible that TRPV1 and TRPA1 channels can form a heterodimer in sensory neurons during basal conditions, and both are able to cross regulate each other's activity (cross-sensitization/desensitization) during inflammation (Gouin et al., 2017). This suggests that TRPA1 likely acts by modulating neuronal excitability, possibly via the regulation of TRPV1 activity.

In addition, spontaneous nocifensive behaviours were also significantly enhanced with TRPA1 deletion or inhibition. This is in contrast to the previous observation for the role of TRPA1 in pruritus (Liu et al., 2013; S. R. Wilson et al., 2013) as well as in nociception (Chen et al., 2011; Fernandes et al., 2013). However, this is unsurprising in the present model, as the enhanced nocifensive behaviours correlate with the increase in skin pathology.

Whilst the pro-inflammatory effects of TRPA1 activation are well established, there is emerging evidence for its protective effects. Recently, capsazepine (CPZ), originally classified as a TRPV1 antagonist (Bevan et al., 1992), has been shown to protect the development of experimental colitis via TRPA1 agonism (Kistner et al., 2016), while TRPA1 deletion was shown to enhance inflammatory responses in various colitis animal models (Bertin et al., 2016; Kun et al., 2014). These results imply that the non-neuronal TRPA1 activation has a major anti-inflammatory activity in contrast to the pro-inflammatory contribution of sensory nerves.

While the main mechanism of action for IMQ is considered to be via TLR7 on cutaneous macrophages and DDC in mice (Hemmi et al., 2002), various TLR7-independent pathways have also been proposed (Kanneganti et al., 2006). To our knowledge here we present the first evidence that IMQ has TRPA1 agonist activity. It induced dose-dependent  $\text{Ca}^{2+}$  influx in TRPA1 transfected CHO cells and a mainly outwardly rectifying transmembrane current on HEK293T cells overexpressing recombinant human TRPA1, and this response was selectively inhibited by the TRPA1 antagonists A967079 and HC030031. By comparison, IMQ did not induce  $\text{Ca}^{2+}$  influx in CHO cells transfected with recombinant TRPV1, indicating that it is unlikely that IMQ would directly influence the functions of cutaneous nociceptors via TRPV1. Thus, IMQ, as a TRPA1 agonist, may potentially activate neural and non-neural cells by their TRPA1 channels, and exert anti-inflammatory activity in the skin.

As TRPA1 KO mice showed a more prominent increase in Th1-associated cytokines, it was proposed that TRPA1 may have an important role in regulating CD4<sup>+</sup> T-cell signalling. Indeed, Bertin *et al* previously showed an interaction between TRPA1 and TRPV1 in CD4<sup>+</sup> T-cell activation towards the Th1 phenotype (Bertin et al., 2016). We found that TRPA1 is also co-localized in CD4<sup>+</sup> T cells in IMQ-induced skin inflammation, in support for Bertin *et al*, but, in contrast to the CD4<sup>+</sup> T cells in the gut, we did not detect TRPV1 expression on skin infiltrating CD4<sup>+</sup> cells. This indicates that this mechanism is unlikely to have major involvement here. Furthermore we did not detect any difference in the number of dermal CD4<sup>+</sup> T cells between TRPA1 wild type and KO animals.

Inflammatory mediators are known to regulate TRPV1 activity via a range of pathways, including specific G-protein coupled receptors (GPCR) (Gouin et al., 2017). Of note, cutaneous denervation in the KC-Tie2 psoriasisform mouse model also results in improvements in acanthosis, decreases in CD4<sup>+</sup> T cells and an elimination of CD11c<sup>+</sup> cells concomitant with decreased IL-23 protein expression, and this effect was suggested to be

mediated by nerve derived SP (substance P) and CGRP (calcitonin gene-related peptide) (Ostrowski et al., 2011). Potentially, IMQ, similarly to CPZ and isopetasin, may directly activate TRPA1 on sensory neurons, leading to the partial desensitization of these nociceptors to TRP-mediated (and perhaps other) stimuli in psoriasis. This, in turn, may result in reduced release of neuropeptides from nerve endings, leading to attenuated DDC activation, decreased IL-12 or IL-23 release, and diminished subsequent generation of Th1 or Th17 T cells, and cutaneous inflammation. Potentially supporting this is our observation that the nocifensive behaviours associated with the early IMQ treatments at day 1 in the mice were not observed in TRPA1 KO or antagonist treated mice (see Fig. 3 a,e), indicating that the TRPA1 channel is indispensable for the early IMQ-evoked pruritus. This phenomenon was very similar in the colitis model, where the first CPZ administration caused pain in the WT mice, while TRPA1 knockouts did not show pain-related behaviour (Kistner et al., 2016). The more intensive nociceptive behaviour at the later time points in the TRPA1 KO mice in the IMQ-induced dermatitis model provides further evidence for a protective phenotype of TRPA1.

In conclusion, to our knowledge these results are the first to show that TRPA1 has a protective role in IMQ-induced psoriasiform dermatitis. We provide evidence that IMQ can directly activate cells through TRPA1 (but not TRPV1). Potentially, the use of TRPA1 agonists may lead to further inhibition of psoriasis-like inflammation. Mustard oil (marketed as a natural anti-psoriasis remedy), and mustard seed, which contains the TRPA1 agonist allyl-isothiocyanate, were recently shown to reduce IMQ inflammation (Yang et al., 2013). Thus there is some evidence to support this strategy. Further studies are required, however, to fully characterize and exploit the pro- and anti-inflammatory potential of the TRPA1 receptor in cutaneous inflammatory reactions.

## ***Materials and Methods***

### ***Full methods are in the Online Supplement***

#### ***Animals***

Animal experiments were performed in Hungary and UK, according to the ARRIVE (Animal Research: Reporting In Vivo Experiments) guidelines, in accordance with the Hungarian and UK law and ethics approval. Female and male TRPV1 wild-type, TRPA1 wild-type (WT, +/+), TRPV1 receptor gene knockout (KO, -/-), TRPA1 KO, and TRPA1/V1 KO mice (8-10 weeks, 20-25 g) were used with respective background strains.

#### ***Induction of psoriasiform skin inflammation***

Psoriasiform dermatitis was induced by Aldara™ cream (5% imiquimod, IMQ, Meda Pharma, Hungary) and vaseline, for vehicle control, using two techniques administered daily for 4 days. Dorsal skin was shaved and depilated before application. The first technique involved the application of 75 mg of Aldara™ on a 4 cm<sup>2</sup> area, known as ‘the whole back model’ as previously characterized (Roller et al., 2012) and was used for nociceptive behaviour experiments. The second model involved a modification of the technique using Finn chambers to enable a more localized application and a lower dose of IMQ in the ‘localized model’. This technique reduced the systemic effects associated with the IMQ model and allowed each mouse to be its own internal control, with lesional and non-lesional skin. For pharmacological studies, the TRPA1 antagonist (A967079; 60mg/kg, p.o.) or vehicle (0.5% methylcellulose in water) was administered twice daily. TRPV1 antagonist (SB366791; 3mg/kg, i.p.) or vehicle (10% DMSO in saline) was administered once daily, 30 minutes before topical skin treatment.

#### ***Measurement of dorsal skin thickness***



Double-fold dorsal skin thickness at the treated areas were measured with micrometers (Moore and Wright, Sheffield, England) with 0.1 mm accuracy.

#### *Measurement of blood flow perfusion changes*

Measurement of dorsal skin blood flow was assessed in the dorsal skin by laser Doppler flowmetry.

#### *Skin scaling score*

The skin scaling score was evaluated and graded 0 to 4 (0: absent to 4: severe).

#### *Radioactive $^{45}\text{Ca}^{2+}$ uptake and $\text{Ca}^{2+}$ influx experiments*

IMQ-induced radioactive  $^{45}\text{Ca}^{2+}$  uptake or  $\text{Ca}^{2+}$  influx was determined on HaCaT (human immortalized keratinocytes) and TRPA1 or TRPV1 receptor-expressing CHO cell lines by scintillation counter or flow cytometry.

#### *Patch clamp recording*

HEK293T cells were seeded on coverslips and whole cell patch clamp measurements were carried out using Axopatch 1.D amplifier and Clampex 10.2 software (Molecular Devices) to record TRPA1 mediated currents.

#### *Nocifensive behavioral studies*

After acclimatization, daily observation was carried out at 4 h after topical skin treatment for 30 minutes. Studies showed 3 typical spontaneous, nocifensive behaviours in IMQ-treated mice: 1) hind paw scratching, 2) biting/licking of the treated area, and 3) flinching, defined as rotational flipping of the dorsal area (Wheeler-Aceto et al., 1990).

### *Histology and immunochemistry*

Skin tissue samples were formalin-fixed (6%) and embedded in paraffin for haematoxylin-eosin or for chloroacetate esterase staining. Scoring parameters and values were determined to evaluate inflammatory alterations. Formalin-fixed paraffin-embedded tissue sections (5  $\mu$ m) were prepared for anti-mouse CD4, TRPV1 and TRPA1 immunohistochemistry.

### *Quantitative RT-PCR*

Dorsal skin samples or dorsal root ganglia (DRG) were collected at various timepoints and were prepared for RT-PCR mRNA profiling of inflammatory cytokine or Iba-1 gene expression as previously described (Sághy et al., 2016).

### *Statistical analysis*

Results are expressed as mean  $\pm$  S.E.M and analyzed statistically using an appropriate test.  $P < 0.05$  accepted as significant.

### **Conflict of interest**

We state hereby that no financial or other relationships exist which might lead to conflicts of interest.

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**References**

- Van Belle a. B, de Heusch M, Lemaire MM, Hendrickx E, Warnier G, Dunussi-Joannopoulos K, et al. IL-22 Is Required for Imiquimod-Induced Psoriasiform Skin Inflammation in Mice. *J Immunol* 2011;188:462–9. doi:10.4049/jimmunol.1102224.
- Bertin S, Aoki-Nonaka Y, Lee J, de Jong PR, Kim P, Han T, et al. The TRPA1 ion channel is expressed in CD4+ T cells and restrains T-cell-mediated colitis through inhibition of TRPV1. *Gut* 2016;66:1584–96. doi:10.1136/gutjnl-2015-310710.
- Bevan S, Hothi S, Hughes G, James IF, Rang HP, Shah K, et al. Capsazepine: a competitive antagonist of the sensory neurone excitant capsaicin. *Br J Pharmacol* 1992;107:544–52. doi:10.1111/j.1476-5381.1992.tb12781.x.
- Chen J, Joshi SK, DiDomenico S, Perner RJ, Mikusa JP, Gauvin DM, et al. Selective blockade of TRPA1 channel attenuates pathological pain without altering noxious cold sensation or body temperature regulation. *Pain* 2011;152:1165–72. doi:10.1016/j.pain.2011.01.049.
- Fernandes ES, Vong CT, Quek S, Cheong J, Awal S, Gentry C, et al. Superoxide generation and leukocyte accumulation: key elements in the mediation of leukotriene B4-induced itch by transient receptor potential ankyrin 1 and transient receptor potential vanilloid 1. *FASEB J* 2013;27:1664–73. doi:10.1096/fj.12-221218.
- van der Fits L, Mourits S, Voerman JS a, Kant M, Boon L, Laman JD, et al. Imiquimod-induced psoriasis-like skin inflammation in mice is mediated via the IL-23/IL-17 axis. *J Immunol* 2009;182:5836–45. doi:10.4049/jimmunol.0802999.
- Flutter B, Nestle FO. TLRs to cytokines: Mechanistic insights from the imiquimod mouse model of psoriasis. *Eur J Immunol* 2013;43:3138–46. doi:10.1002/eji.201343801.
- Gouin O, L'Herondelle K, Lebonvallet N, Le Gall-Ianotto C, Sakka M, Buh?? V, et al. TRPV1 and TRPA1 in cutaneous neurogenic and chronic inflammation: pro-inflammatory

- response induced by their activation and their sensitization. *Protein Cell* 2017;1–18.  
doi:10.1007/s13238-017-0395-5.
- Hemmi H, Kaisho T, Takeuchi O, Sato S, Sanjo H, Hoshino K, et al. Small anti-viral compounds activate immune cells via the TLR7 MyD88-dependent signaling pathway. *Nat Immunol* 2002;3:196–200. doi:10.1038/ni758.
- Jurk M, Heil F, Vollmer J, Schetter C, Krieg AM, Wagner H, et al. Human TLR7 or TLR8 independently confer responsiveness to the antiviral compound R-848. *Nat Immunol* 2002;3:499. doi:10.1038/ni0602-499.
- Kanneganti T-D, Ozören N, Body-Malapel M, Amer A, Park J-H, Franchi L, et al. Bacterial RNA and small antiviral compounds activate caspase-1 through cryopyrin/Nalp3. *Nature* 2006;440:233–6. doi:10.1038/nature04517.
- Kistner K, Siklosi N, Babes A, Khalil M, Selescu T, Zimmermann K, et al. Systemic desensitization through TRPA1 channels by capsazepine and mustard oil - a novel strategy against inflammation and pain. *Sci Rep* 2016;6:28621. doi:10.1038/srep28621.
- Kobayashi K, Fukuoka T, Obata K, Yamanaka H, Dai Y, Tokunaga A, et al. Distinct expression of TRPM8, TRPA1, and TRPV1 mRNAs in rat primary afferent neurons with Aδ/C-fibers and colocalization with Trk receptors. *J Comp Neurol* 2005;493:596–606. doi:10.1002/cne.20794.
- Kun J, Helyes Z, Perkecz A, Bán Á, Polgár B, Szolcsányi J, et al. Effect of Surgical and Chemical Sensory Denervation on Non-neural Expression of the Transient Receptor Potential Vanilloid 1 (TRPV1) Receptors in the Rat. *J Mol Neurosci* 2012;48:795–803. doi:10.1007/s12031-012-9766-9.
- Kun J, Szitter I, Kemény A, Perkecz A, Kereskai L, Pohóczky K, et al. Upregulation of the transient receptor potential ankyrin 1 ion channel in the inflamed human and mouse colon and its protective roles. *PLoS One* 2014;9. doi:10.1371/journal.pone.0108164.

- Liu B, Escalera J, Balakrishna S, Fan L, Caceres AI, Robinson E, et al. TRPA1 controls inflammation and pruritogen responses in allergic contact dermatitis. *FASEB J* 2013;27:3549–63. doi:10.1096/fj.13-229948.
- Nestle FO, Kaplan DH, Barker J. Psoriasis. *N Engl J Med* 2009;361:496–509. doi:10.1056/NEJMra0804595.
- Ostrowski SM, Belkadi A, Loyd CM, Diaconu D, Ward NL. Cutaneous denervation of psoriasiform mouse skin improves acanthosis and inflammation in a sensory neuropeptide-dependent manner. *J Invest Dermatol* 2011;131:1530–8. doi:10.1038/jid.2011.60.
- Perera GK, Di Meglio P, Nestle FO. Psoriasis. *Annu Rev Pathol Mech Dis* 2012;7:385–422. doi:10.1146/annurev-pathol-011811-132448.
- Quivy V, Van Lint C. Regulation at multiple levels of NF-kappaB-mediated transactivation by protein acetylation. *Biochem Pharmacol* 2004;68:1221–9. doi:10.1016/j.bcp.2004.05.039.
- Riol-Blanco L, Ordovas-Montanes J, Perro M, Naval E, Thiriot A, Alvarez D, et al. Nociceptive sensory neurons drive interleukin-23-mediated psoriasiform skin inflammation. *Nature* 2014;510:157–61. doi:10.1038/nature13199.
- Roller A, Perino A, Dapavo P, Soro E, Okkenhaug K, Hirsch E, et al. Blockade of Phosphatidylinositol 3-Kinase (PI3K) $\delta$  or PI3K $\gamma$  Reduces IL-17 and Ameliorates Imiquimod-Induced Psoriasis-like Dermatitis. *J Immunol* 2012;189:4612–20. doi:10.4049/jimmunol.1103173.
- Sághy É, Sipos É, Ács P, Bölcskei K, Pohóczky K, Kemény Á, et al. TRPA1 deficiency is protective in cuprizone-induced demyelination—A new target against oligodendrocyte apoptosis. *Glia* 2016;64:1–15. doi:10.1002/GLIA.23051.
- Schön MP, Schön M, Klotz K-N. The small antitumoral immune response modifier imiquimod interacts with adenosine receptor signaling in a TLR7- and TLR8-independent

- fashion. *J Invest Dermatol* 2006;126:1338–47. doi:10.1038/sj.jid.5700286.
- Swindell WR, Michaels KA, Sutter AJ, Diaconu D, Fritz Y, Xing X, et al. Imiquimod has strain-dependent effects in mice and does not uniquely model human psoriasis. *Genome Med* 2017;9:24. doi:10.1186/s13073-017-0415-3.
- Vinter H, Kragballe K, Steiniche T, Gaestel M, Iversen L, Johansen C. Tumour necrosis factor-alpha plays a significant role in the Aldara-induced skin inflammation in mice. *Br J Dermatol* 2016;174:1011–21. doi:10.1111/bjd.14320.
- Wheeler-Aceto H, Porreca F, Cowan A. The rat paw formalin test: comparison of noxious agents. *Pain* 1990;40:229–38.
- Wilson SR, Nelson AM, Batia L, Morita T, Estandian D, Owens DM, et al. The Ion Channel TRPA1 Is Required for Chronic Itch. *J Neurosci* 2013;33:9283–94. doi:10.1523/JNEUROSCI.5318-12.2013.
- Wilson SRR, Thé L, Batia LMM, Beattie K, Katibah GEE, McClain SPP, et al. The Epithelial Cell-Derived Atopic Dermatitis Cytokine TSLP Activates Neurons to Induce Itch. *Cell* 2013;155:285–95. doi:10.1016/j.cell.2013.08.057.
- Yang R, Zhou Q, Wen C, Hu J, Li H, Zhao M, et al. Mustard seed (*Sinapis Alba* Linn) attenuates imiquimod-induced psoriasiform inflammation of BALB/c mice. *J Dermatol* 2013;40:543–52. doi:10.1111/1346-8138.12119.

## Figure legends

**Figure 1:** *Effect of topical Aldara treatment on skin thickness, blood perfusion and skin scaling response in TRPA1 WT and KO mice and A967079-treatment. a, c:* 75 mg Aldara was applied to the back skin of TRPA1 WT animals. A967079 or vehicle (0.5% methylcellulose in water) was administered orally at 30 minutes and 5 hours after topical skin treatment (**c**). **B:** 25 mg vaseline (V) or Aldara (A) was applied to the shaved back skin of TRPA1 WT and KO animals using Finn chambers. **d, e, f:** Percent change in skin thickness after vaseline or Aldara treatment in TRPA1 WT or KO male mice (**d**) and TRPA1 WT or KO female animals using Finn chamber (**e**) or A967079 pre-treated TRPA1 WT male mice (**f**). **g, h, i:** Percent change in dorsal skin blood flow after vaseline or Aldara treatment in TRPA1 WT or KO (**g**) and TRPA1 WT or KO animals using Finn chamber (**h**) or after A967079 pre-treatment in TRPA1 WT mice (**i**). **j, k, l:** Skin scaling PASI scores after vaseline or Aldara treatment in TRPA1 WT or KO (**j**) and TRPA1 WT or KO animals using Finn chambers (**k**) or in A967079 pre-treated TRPA1 WT mice (**l**). Data are mean  $\pm$  SEM for n = 6-12/group. \*p < 0.05; \*\*p < 0.01; \*\*\*p < 0.001 vaseline vs. Aldara-treated sites, #p<0.05; ##p<0.01; ###p<0.001 Aldara-treated wild-type vs. Aldara-treated KO or Aldara-treated WT/Veh vs. Aldara-treated knockout/A967079-treated group, based on repeated measures 2-way ANOVA followed by Bonferroni's *post hoc* test.

**Figure 2.** *Representative histological view of TRPA1 wild-type and TRPA1 KO mouse dorsal skin following Aldara-treatment. Panel a* upper line: vaseline-treated control skin of TRPA1 WT and KO animal at 100x magnification (scale bar=200  $\mu$ m). *Panel a* middle line: Aldara-treated dorsal skin tissue of TRPA1 WT and KO mouse at 200x magnification (scale bar=200  $\mu$ m). *Panel a* bottom line: Aldara-treated dorsal skin tissue of TRPA1 WT and KO mouse (400x, scale bar=100  $\mu$ m). PK: parakeratosis, HK: hyperkeratosis, MM: Munro's



microabscesses **b**: Arbitrary score of vaseline- or Aldara-treated dorsal skin samples of TRPA1 WT and KO animals. **c**: Arbitrary score of vaseline- or Aldara-treated dorsal skin samples of TRPA1 WT animals pre-treated with A967079 or its vehicle. Data are mean  $\pm$  SEM for  $n = 4-7/\text{group}$ .  $^{**}p < 0.01$ ;  $^{***}p < 0.001$  vaseline vs. Aldara-treated sites,  $\#p < 0.05$  Aldara-treated wild-type vs. Aldara-treated knockout group or vehicle+Aldara-treated vs. A967079+Aldara-treated group, based on 2-way ANOVA followed by Bonferroni's *post hoc* test.

**Figure 3.** *Repeated TRPA1 inhibition resulted in increased spontaneous nocifensive behaviours.* 75 mg Aldara was applied to the back skin of male TRPA1 WT or KO animals. A967079 or vehicle (0.5% methylcellulose in water) was administered orally at 30 minutes and 5 hours after topical skin treatment. Behavioural observation was performed daily at 4 hours following topical skin treatment, for 30 minutes. Mice were acclimatized to the setup for 2 days (days -3 and -2). **a**) day 4 spontaneous behaviours, **b**) hind paw scratching, **c**) biting/licking of treated dorsal skin, **d**) flinching of the dorsal region in TRPA1 WT or KO animals. **e**) day 4 spontaneous behaviours, **f**) hind paw scratching, **g**) biting/licking of treated dorsal skin, **h**) flinching of the dorsal region in vehicle or A967079 pre-treated animals. Data are mean  $\pm$  SEM for  $n = 5-7/\text{group}$ .  $^{*}p < 0.05$ ;  $^{**}p < 0.01$ ;  $^{***}p < 0.001$  vaseline vs. Aldara-treated sites,  $\#p < 0.05$ ;  $\##p < 0.01$ ;  $\###p < 0.001$  Aldara-treated Veh vs. Aldara-treated A967079 groups, based on repeated measures 2-way ANOVA followed by Bonferroni's *post hoc* test.

**Figure 4.** *Imiquimod-induced relative expression of distinct inflammatory cytokines in TRPA1 WT and KO mice at different time points.* **a**: IL-1 $\beta$  relative quantity, **b**: TNF- $\alpha$  relative quantity, **c**: IL-23 relative quantity, **d**: IL-17 relative quantity, **e**: IL-22 relative quantity

measured with RT qPCR. Data are mean  $\pm$  SEM for n = 5/group. \*p < 0.05; \*\*\*p < 0.001 vaseline vs. Aldara-treated sites, ##p < 0.01; ###p < 0.001 Aldara-treated wild-type vs. Aldara-treated knockout group, based on student t-test.

**Figure 5:** *Effect of TRPA1 and TRPV1 loss of function on Aldara-mediated skin inflammation*

*(a, b, c, d, e, f). Imiquimod-induced  $Ca^{2+}$  influx in TRPA1-, TRPV1-expressing CHO cells or*

*HaCaT cell lines (g, h). a, b:* 25 mg vaseline or Aldara was applied to the shaved back skin of

TRPV1 WT and KO animals in Finn chambers. Percent change of skin thickness **(a)** and

blood perfusion **(b)** were compared to the vaseline-treated groups. **c, d:** 75 mg Aldara was

applied to the back skin of TRPA1 WT or KO animals. SB366791 TRPV1 receptor antagonist

(3mg/kg, *i.p.*) or its vehicle (Veh, 10% DMSO in saline) was administered 30 minutes prior to

Aldara-treatment. Percent change of skin thickness **(c)** and dorsal skin blood flow **(d)** was

monitored as described in the methods section. **e, f:** 25 mg vaseline or Aldara was applied to

the shaved back skin of TRPV1-, TRPA1 or TRPA1/V1 WT and KO animals in Finn

chambers. Percent change of skin thickness **(e)** and blood perfusion **(f)** were compared to the

Aldara-treated wild-type groups of each mice strains. Data are mean  $\pm$  SEM for n = 6-

12/group. \*p < 0.05; \*\*p < 0.01; \*\*\*p < 0.001 vaseline-treated vs. Aldara-treated groups or

Aldara-treated TRPA1 WT vs. Aldara-treated TRPA1 KO+vehiculum (veh) groups, #p <

0.05; ##p < 0.01; ###p < 0.001 Aldara-treated TRPV1 WT vs. Aldara-treated TRPV1 KO

groups (A,B) or Aldara-treated TRPA1 KO+vehiculum (Veh) vs. Aldara-treated TRPA1

KO+SB366791 groups **(c,d)** or TRPA1 KO vs. TRPV1- and double KO animals **(e,f)**, based

on repeated measures 2-way ANOVA followed by Bonferroni's *post hoc* test. **g:** Dose-

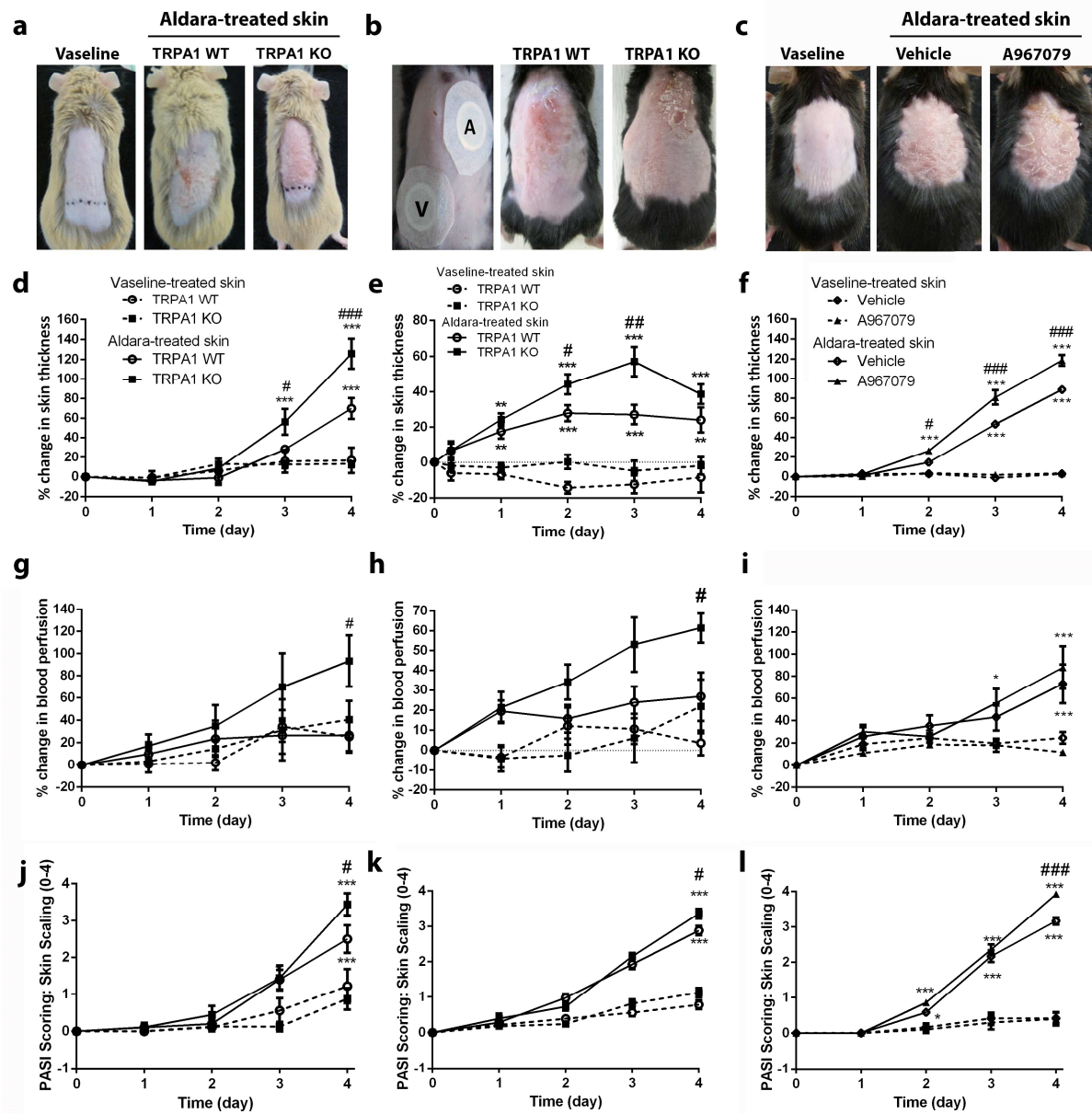
response curve induced by IMQ in TRPA1 or TRPV1 expressing CHO cells or in HaCaT cell

line monitored by radioactive  $^{45}Ca^{2+}$  uptake. **h:** IMQ-induced Fluo-4 signal ratio in TRPA1+

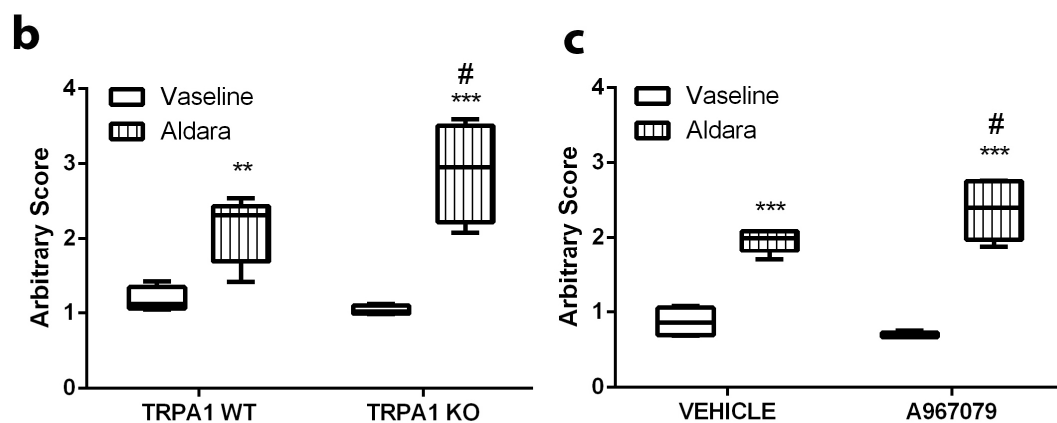
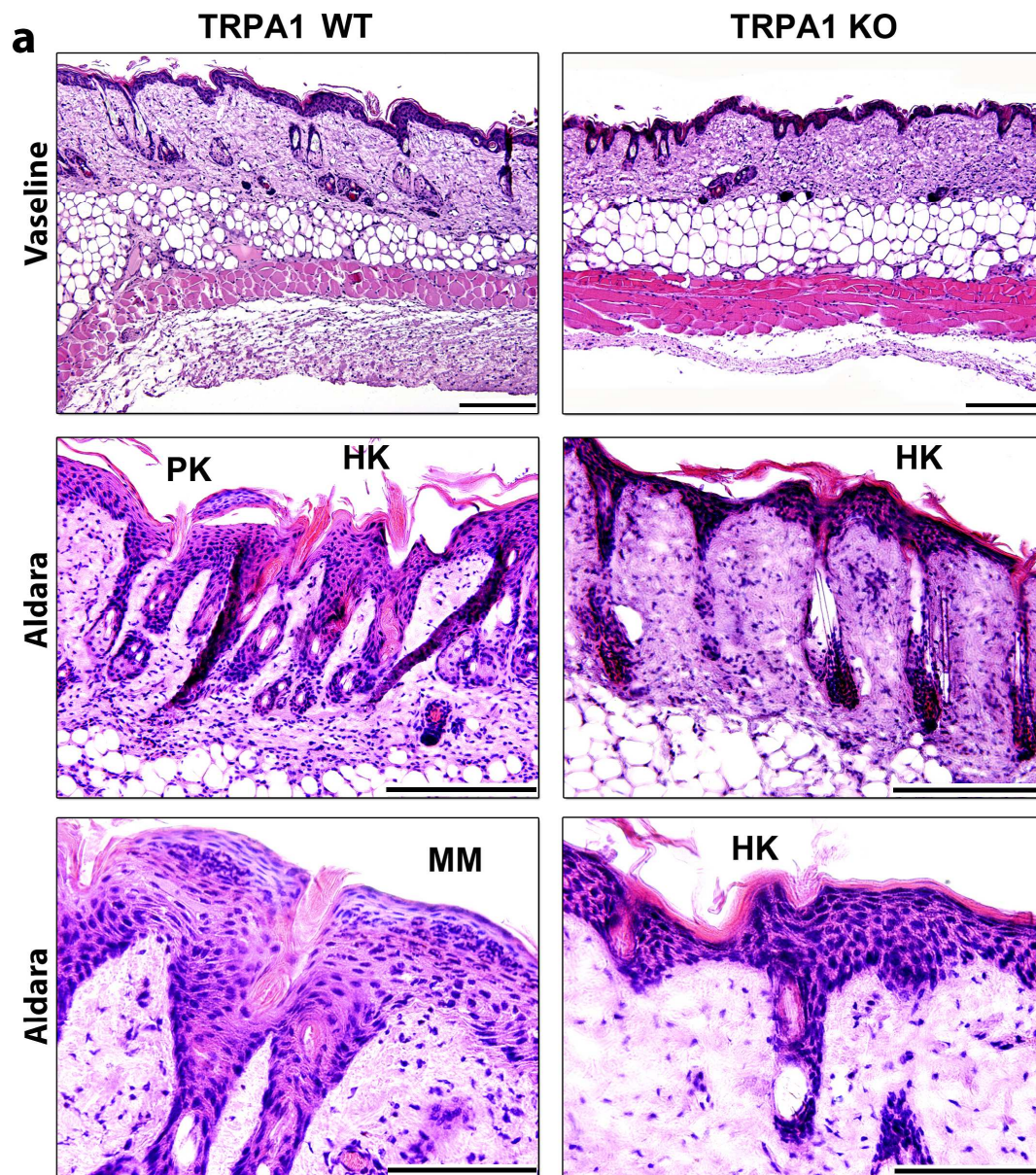
CHO cells after mustard oil (MO), imiquimod (IMQ) or different concentrations of A967079

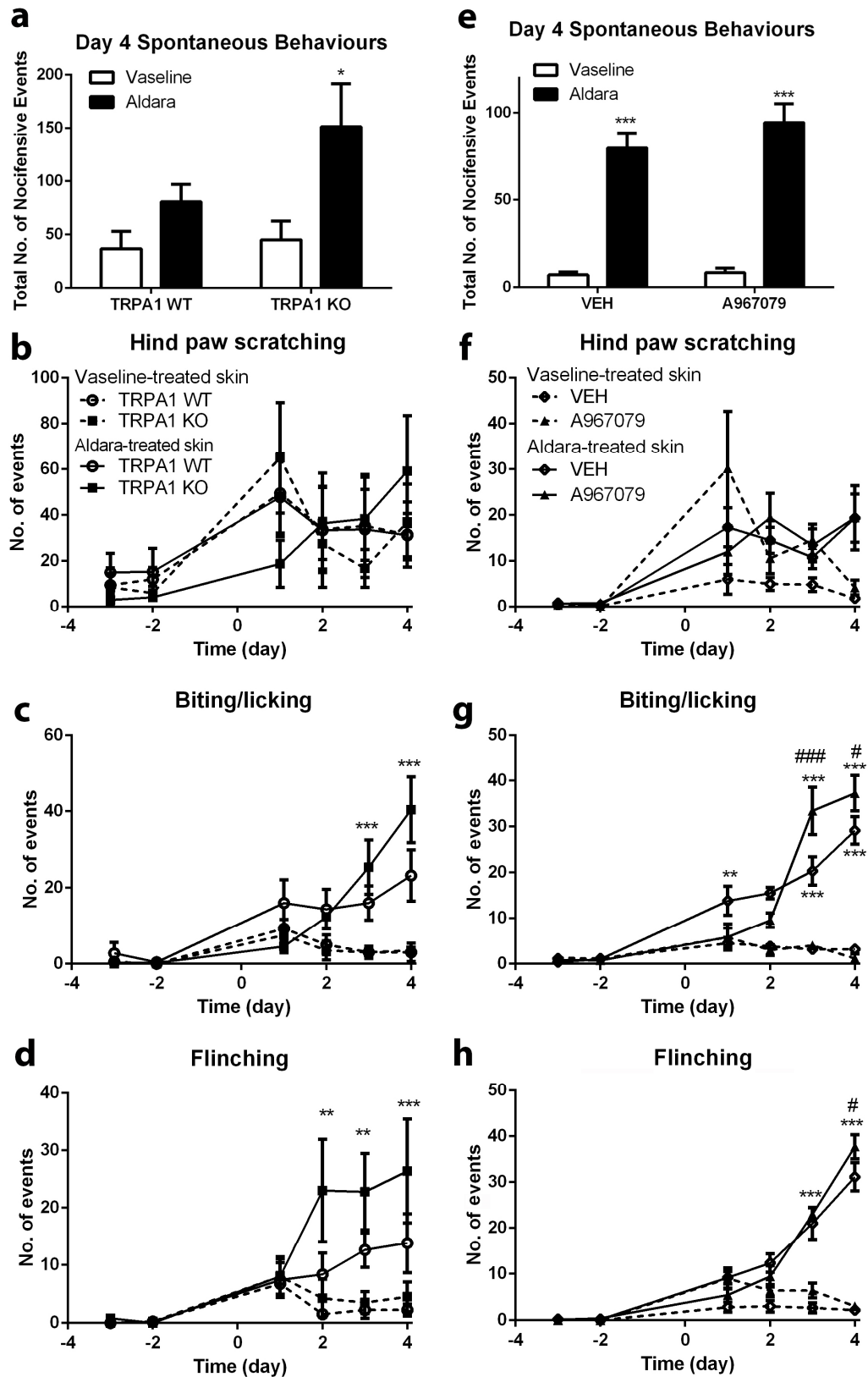
(A96) treatments, \*\*\* $p < 0.001$  A96+50  $\mu\text{M}$  IMQ vs. 50  $\mu\text{M}$  IMQ treated groups based on unpaired t test.

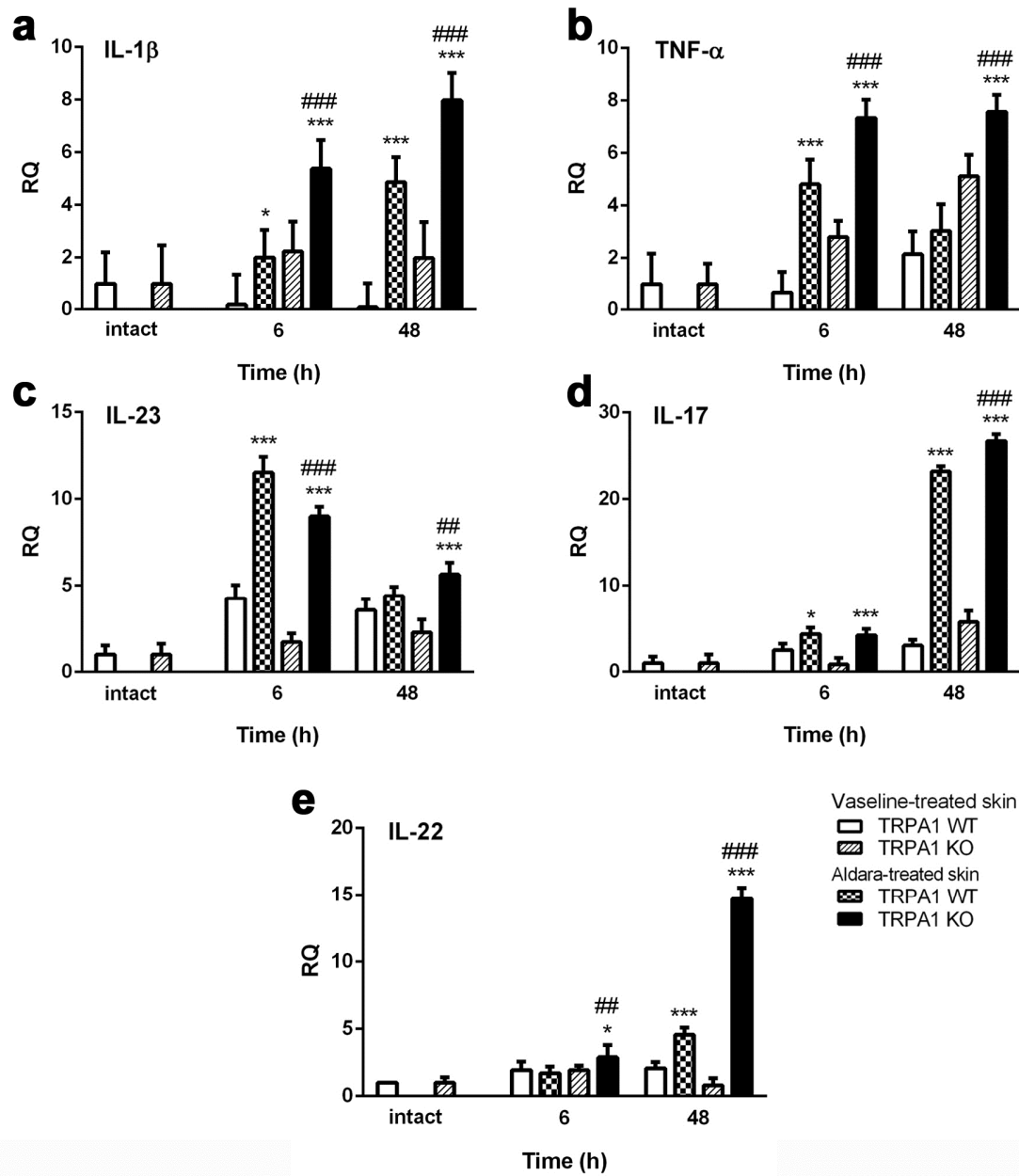
**Figure 6.** *Immunohistological detection of TRPA1+/CD4+ T helper cells or TRPV1+/CD4+ T helper cells in IMQ-treated mouse dorsal skin samples. Single optical section have been taken using a 40x fluorescent objective with phase contrast capacity (scale bar=100  $\mu\text{m}$ ).*

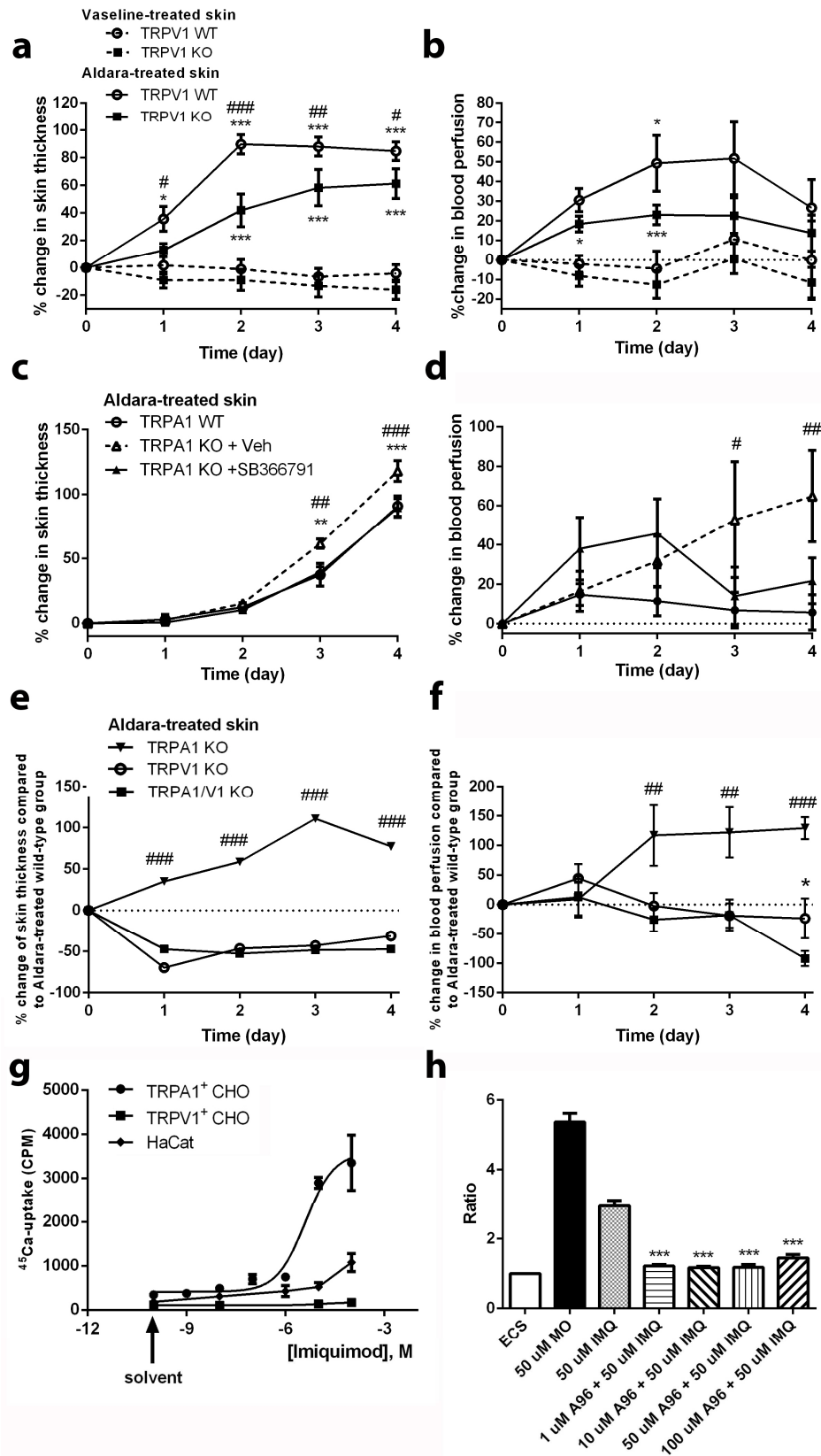




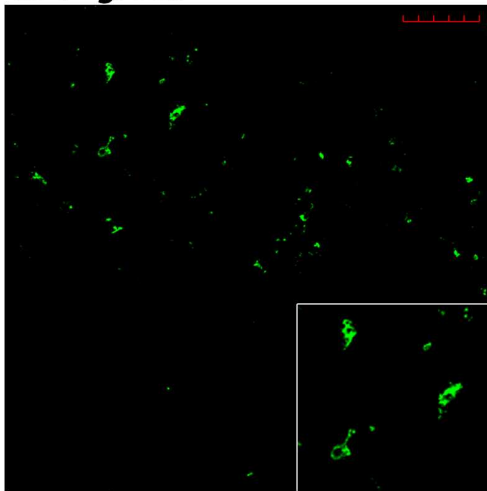
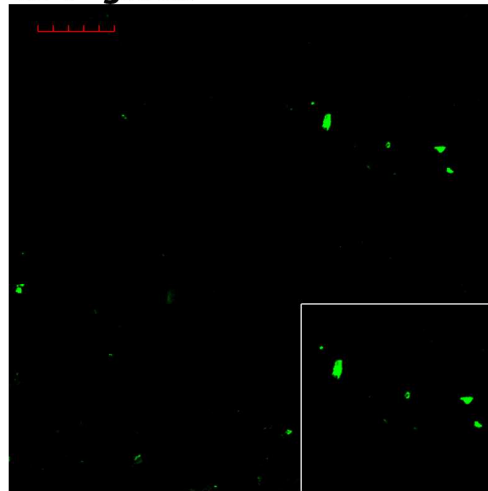
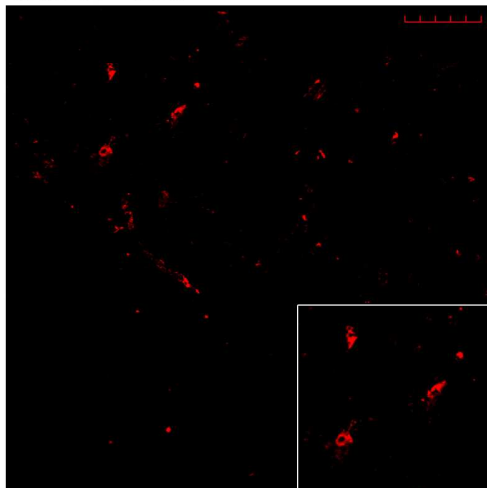
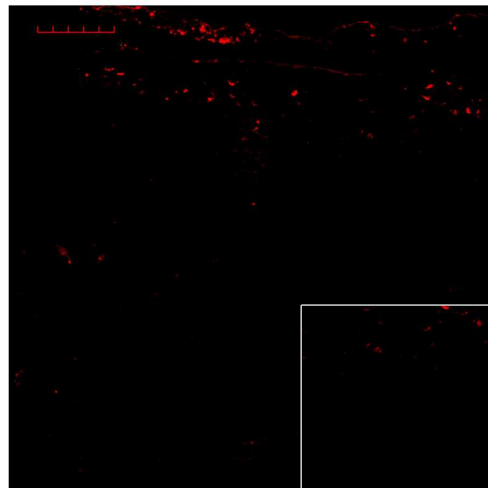
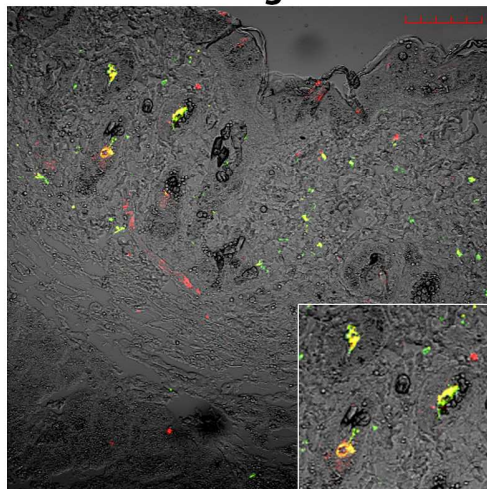










**CD4 - green****CD4 - green****TRPA1 - red****TRPV1 - red****CD4/TRPA1 merged****CD4/TRPV1 merged**